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Short communication

An aptamer-based chromatographic strip assay for sensitive toxin semi-quantitative detection

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ABSTRACT

An aptamer-based chromatographic strip assay method for rapid toxin detection was developed. The aptamer-based strip assay was based on the competition for the aptamer between ochratoxin A and DNA probes. The sensing results indicated that the sensitivity of the aptamer-based strip was better than that of conventional antibody-based strips. The visual limit of detection of the strip for qualitative detection was 1 ng/mL while the LOD for semi-quantitative detection could down to 0.18 ng/mL by using scanning reader. The recoveries of test samples were from 96% to 110%. All detections could be achieved in less than 10 min, indicating that the aptamer-based strip could be a potential useful tool for rapid on-site detections.

1. Introduction

Ochratoxin A (OTA), a type of mycotoxin, is mainly produced by fungal species in Aspergillus and Penicillium genera (Khoury and Atoui, 2010). Studies show that OTA is hepatotoxic, nephrotoxic, neurotoxic, teratogenic and mutagenic to most of the mammalian species (Clarke et al., 1993). What makes things worse is that OTA could contaminate many foods and drinks, such as cereals, nuts, coffee, beer and wine (Cruz-Aguado and Penner, 2008; Ahmed et al., 2007). And OTA is reported to exist in human blood and mother's milk (Gareis et al., 1988). Recently, OTA has been detected worldwide in various foods and feed sources. Therefore, it is of great importance to develop the rapid and sensitive methods for OTA detection to ensure food safety issues and guarantee human health. The European Commission Regulation (EC) required that OTA in grape juices, wines (red, white and rose) should be less than 2 ng/mL (Andreel and Ali, 2010). The instrument-based methods such as high performance liquid chromatography (HPLC) and fluorescence polarization immunoassay (FPIA) are widely accepted as the classic methods that have good accuracy and reproducibility (Zezza et al., 2009; Blesa et al., 2004) and with the sensitivity down to 0.01 ng/mL and 2 ng/mL, respectively. However, these instrumental methods could not meet the requirements of on-site detections due to the expensive equipments and trained personnel. Some rapid detection methods are further designed for the rapid and on-site screening and detection. Both enzyme linked immunosorbent assay (ELISA) and colloid gold nanoparticle based immunochromatographic strip are the typical rapid on-site detection methods and with the limit of detection (LOD) down to 2 ng/mL and 5 ng/mL, respectively (Liu et al., 2008; Ildiko et al., 1996). Meanwhile the antibody modified nanomaterials for the sensitive OTA detections were also reported with the LOD at the level of less than 1 ng/mL (Ma et al., 2009; Wang et al., 2010). Note that all these series methods are both heavily relied on the quality of the used antibodies. Usually, it takes a long time to immune the animal and to obtain the corresponding useful antibody (Baldrich et al., 2005; Liu et al., 2010). And it is also generally accepted that it is of difficulty to get the antibody against the small chemical molecules including certain toxins.

Aptamers, which are single stranded oligonucleotides of specific sequences, can be selected from a pool of DNAs or RNAs by the classic systematic evolution of ligands by exponential enrichment (SELEX) process (Tuerk and Gold, 1990; Ellington and Szostak, 1990; Liu et al., 2009). Aptamers have high specificity to different targets including proteins (Lin et al., 2006; Ho and Leclerc, 2004; Bang et al., 2005), metal ions (Smironov and Shafer, 2000), and organic molecules (Stojanovic and Landry, 2002). Aptamers have been widely used to replace antibodies in detection system because of their high specificity, easy and reproducible production and easy

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of modification for applications. The aptamer-based electrochemical method for the OTA detection has already been reported by our group (Kuang et al., 2010; Wang et al., 2009; Zhang et al., 2010). For further research focused on the aptamer based detections, herein, we report the OTA detection using an aptamer-based strip. Aptamer modified gold nanoparticles were used as the visual reporter and the aptamer-based strip was used to detect OTA successfully. The specificity and stability of the fabricated strip were also evaluated and rapid OTA detection in real samples was also demonstrated.

2. Experimental

2.1. Materials and reagents

OTA, $HAuCl_4 \cdot 3H_2O$, Polyvinylpyrrolidone (PVP) and sodium citrate were purchased from the Sigma–Aldrich and used directly without further purification. N-hydroxy-succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were from J&K Chemical Ltd. The nitrocellulose high-flow plus membranes and glass fiber membranes were from Whatman (Dassel, Germany). Semi-rigid polyethylene sheets and adhesive tape were purchased locally in Wuxi, Jiangsu.

Thiol-modified aptamer and other two biotin-modified DNA probes were obtained from Sangon Biotech (Shanghai) Co., Ltd. The detailed sequence of the aptamer and two DNA probes were:

Aptamer: 5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA AAA AAA AAA AAA AAA AAA-SH-3′
Test line DNA probe 1: 5′-Biotin-CTA GCC CAC ACC CAC CGC ATT TCC CTC GTA GCC TGT-3′
Control line DNA probe 2: 5′-Biotin-TTT TTT TTT TTT TTT TTT-3′

2.2. Preparation of the gold nanoparticles (GNPs)

The preparation of the GNPs was according to the classic Frens method with little modification. First, all glassware for the preparation was thoroughly cleaned with aqua regia (HCl/HNO_3, volume ratio 3:1) and oven-dried for use. Typically, 100 mL of 0.01% $HAuCl_4$ solution was boiled thoroughly, and 1.8 mL of 1% trisodium citrate solution was added rapidly under constant stirring. The solution was boiled for another 5 min when the color of the solution changed from blue to wine-red in 1 min. After cooling at room temperature with stirring, the solution was stored at 4 °C in dark for further study. The size distribution of the prepared GNPs was confirmed by the transmission electron microscopy (Figure S1).

2.3. Preparation of GNPs–aptamer probe

The preparation of GNPs–aptamer conjugations was based on the formation of thiol–gold bond. Briefly, before the conjugation, the GNPs solution was centrifugated at 10,000 rpm for 15 min and re-dispersed to a specific concentration with sterile distilled water. Thiol modified aptamer solution (10 μM) was added into the GNPs solution (volume ratio, 1:1) and reacted at room temperature for 8 h. Then, a 2 M NaCl solution was added to a final concentration of 50 mM and reacted for another 12 h. The mixture was purified by centrifugation at 6500 rpm for 15 min. The GNP–aptamer conjugation solution was stored at 4 °C for further study. Confirmation of conjugation was carried out by the agarose gel electrophoresis (see Figure S2 in supporting information).

2.4. Preparation of streptavidin–biotin–DNA probe conjugates

Streptavidin was dissolved in the 0.01 mM phosphate-buffered saline (PBS) solution (pH 7.4) at 1 mg/mL. 5 μL streptavidin solution and 35 μL 5 μM DNA probe solution were mixed and reacted at 4 °C for 2 h. The mixture was centrifugated at 6000 rpm with a centrifugal filter (cutoff 30,000, Millipore) for 30 min to remove excess DNA probes. After two times washes with the PBS solution, the streptavidin–biotin–DNA probe conjugates were dissolved in the PBS solution to the previous volume and used for following control and test lines fabrication of the strip.

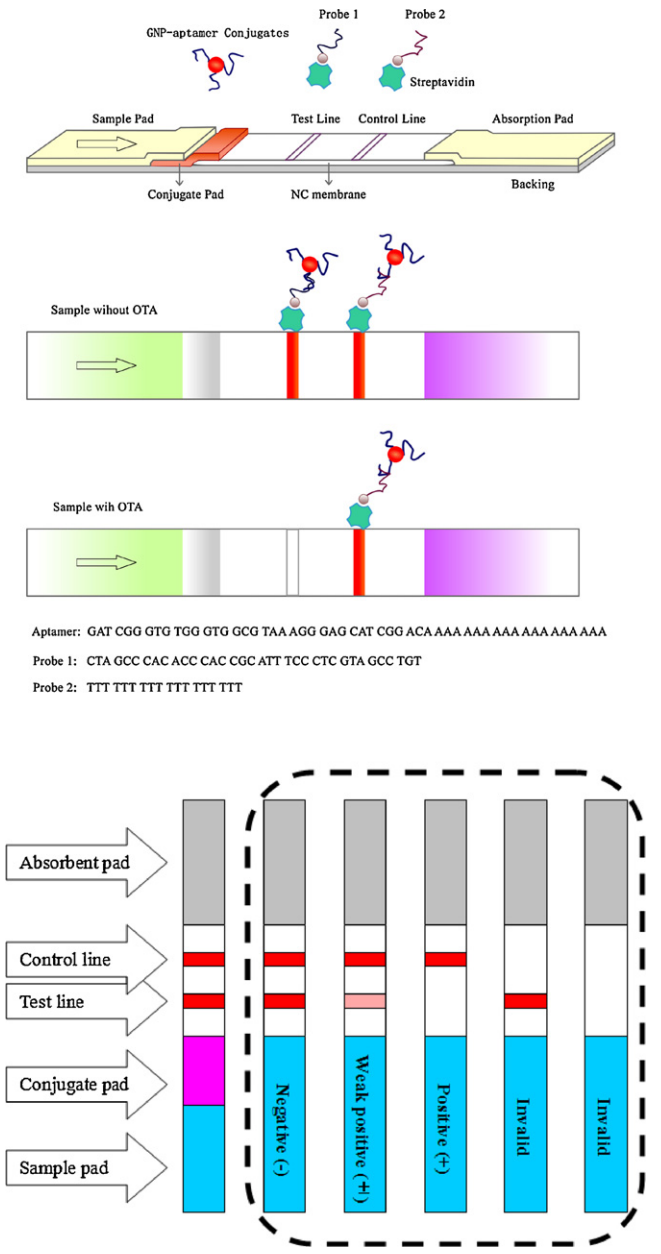

Fig. 1. Schematic diagram of the principle for the detection of aptamer-based strip.

2.5. Fabrication of the aptamer-based strip

The schematic of the strip fabrication is shown in Fig. 1. The nitrocellulose membrane, glass-fiber membrane (conjugated pad), sample pad and absorbent pad were laminated 2 mm with each other in sequence and pasted onto the plastic back plate. The width of the plate was cut into 4 mm. The streptavidin–DNA probe 1 and streptavidin–DNA probe 2 conjugates were dispersed on the nitrocellulose membrane as test line and control line, respectively. The strips were dried at room temperature for 10 min. GNP–aptamer probe (5 μL/strip) was added to the glass fiber membrane and air-